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Winter decomposition of transgenic cotton residue in conventional-till and no-till systems

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Abstract

Current research suggests that genetic modification of commercial crops may lead to indirect effects on ecosystem function (i.e. decomposition and nutrient cycling processes). We investigated residue decomposition of cotton that was genetically modified to express an endotoxin insecticide isolated from *Bacillus thuringiensis* (*Bt*) and/or glyphosate tolerance (Roundup Ready®). Decomposition of the genetically modified residue was compared within agricultural systems under conventional-tillage (CT) or no-tillage (NT) management. We tested for variation in decomposition dynamics under the two tillage regimes because there are intrinsic differences in environmental and biotic conditions between them, and that both management methods are employed in cotton production. We hypothesized that decomposition dynamics would be affected by the presence or absence of the *Bt* endotoxin and that the degree of variation would be more distinct between tillage regimes. Decomposition dynamics were determined by change in mass remaining and nutrient content (C and N) of cotton litter material contained in mesh litterbags collected over a 20-week period from December to May. Rate of decomposition and change in nutrient content of decomposing litter within either tillage regime was not significantly different between the two cotton types examined. Percent mass remaining, total N and total C decreased over time and were significantly different between tillage regimes only. Over the 20-week experiment, mass loss with subsurface decomposition in the CT reached 55% but surface decomposition in the NT reached only 25%. We observed that cotton genetically modified to express *Bt* endotoxin and glyphosate tolerance decomposed similarly to cotton modified for glyphosate tolerance only.

Keywords: *Bacillus thuringiensis*; Genetically modified organism; Nutrient dynamics; Conservation tillage; Litterbag method

1. Introduction

Genetically modified organisms (GMOs) have been used in the agricultural environment prior to establishing direct and/or indirect effects of their release on ecosystem function. Tomlin (1994) proposed that non-target organisms such as earthworms might be susceptible to pesticides engineered into agricultural crops. Although engineered pesticides such as *Bt* toxin released by root exudation accumulated and remained biologically active in the soil (Saxena and Stotzky, 2001a), availability of *Bt* has had little direct effect (i.e. lethal or reproductive) on specific non-target soil organisms such as the collembolan *Folsomia candida*, and earthworm *Lumbricus ter-*

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restris (Yu et al., 1997; Saxena and Stotzky, 2001a). With the lack of established direct effects, reason exists to focus on non-direct effects that could alter ecosystem function. Morra (1994) and Trevors et al. (1994) proposed that release of GMOs could affect nutrient cycles through negative interactions with soil microbes by altering microbial species composition or affecting microbial physiology. Microbial counts, species composition and substrate utilization patterns were altered in the presence of cotton leaf material expressing the Cry1A(c) endotoxin isolated from *Bacillus thuringiensis* (*Bt*) var. *kurstaki* (Donegan et al., 1995). Microbial species composition, determined by Biolog GN metabolic fingerprinting, was different between the rhizospheres of parental and transgenic alfalfa (Di Giovanni et al., 1999). Similarly, diversity of bacteria associated with roots of canola that expressed glyphosate tolerance was different than related non-transgenic cultivars (Siciliano and Germida, 1999).

Ecosystem function, specifically decomposition and nutrient cycling processes, might be affected by plant genetic alteration through changes in physiological traits that can affect soil organisms. Organic matter decomposition and nutrient cycling is regulated by decomposer organisms, resource quality, and the physico-chemical environment (Swift et al., 1979; Heal et al., 1997). Decomposer community composition (i.e. food web structure), influences decomposition dynamics (Wardle and Lavelle, 1997; Neher, 1999). Observed changes in microbial dynamics (i.e. species composition, numbers, and substrate use), in the presence of genetically altered plants (Donegan et al., 1995; Di Giovanni et al., 1999) could impact the decomposer food web. Differences in decomposition dynamics between transgenic and parent line tobacco plants was linked to the response of decomposer organisms to variation in resource quality (measured by carbon content) (Donegan et al., 1997). Changes in plant characteristics, apart from the direct expression of the gene product, that affect resource quality were reported by Saxena and Stotzky (2001b) who determined *Bt* corn had a higher lignin content than non-*Bt* corn. Change in carbon or lignin content might affect decomposition dynamics. As measures of resource quality, carbon to nitrogen ratio (C:N), and lignin to N ratio (L:N), have been used to predict rates of decomposition (Heal et al., 1997). For instance, organic materials with high C:N or high L:N are expected to decompose at slower rates than materials with low C:N. Physico-chemical environment can shape the decomposer community. Soil microbial biomass in conservation tillage (no-till; NT) systems was dominated by fungi, whereas conventional-tillage (CT) systems were dominated by bacteria (Hendrix et al., 1986; Beare, 1997; Frey et al., 1999). The physico-chemical environment to which organic matter is exposed in these tillage systems is different because crop residues in no-till systems decompose on the soil surface whereas under conventional-tillage, residues are incorporated and exposed to the subsurface decomposer food webs. Whether by modification of the decomposer community or by change in resource quality, genetically modified crops are predicted to alter decomposition dynamics and nutrient cycling. How dynamics will be affected within the contrasting physico-chemical environments of conservation or conventional-tillage systems in relation to their fungal versus bacterial components has not been evaluated.

We initiated research on the effects of genetically modified crops on the environment by planting two commercially available lines of cotton both expressing glyphosate tolerance and one expressing the endotoxin insecticide isolated from *Bt*. We hypothesized that an alteration in resource quality due to changes in the physiological traits of the plant would result in differences in the decomposition dynamics of plant residues. We tested for differences in decomposition under both conventional-tillage and no-tillage regimes in order to analyze effects under contrasting environmental conditions and decomposer communities (Beare et al., 1992).

2. Materials and methods

The Horseshoe Bend (HSB) experimental site is a long-term project funded under the National Science Foundation Long-term Research in Environmental Biology program. The experimental design is completely randomized for two tillage management regimes, conventional-tillage and no-tillage, overlaid with a split plot arrangement for two Roundup Ready® cotton varieties, Sure-gro 125RB, expressing both glyphosate tolerance and the *Bt* toxin (RR+Bt), and Sure-gro 125R, expressing only glyphosate toler-

ance (RR−Bt). The CT and NT plots were established in 1978; the cotton split plots were established in spring 1999. Three replicate NT and three replicate CT plots were used for the litterbag decomposition study. After the cotton-growing season, all plots were mowed. The conventional-tillage plots were also moldboard plowed, disked and rotary-tilled. All plots were planted with a cover crop of winter rye with a broadcast seeder. Physical and chemical properties of these soils have been described by Beare et al. (1992) and references therein.

Leaves and stems from cotton were collected from whole plants harvested at the end of the first growing season. Litterbags, 15 cm × 15 cm, were constructed of black nylon window screen with a mesh ca. 1.7 mm. Five grams (oven-dry weight equivalent at 50 °C) of leaf and stem (3:1) were used in each bag. "Traveler bags," taken to the field site and returned to the laboratory, were used to estimate losses due to transportation. Litterbags with cotton materials were placed in their respective treatments, RR−Bt or RR+Bt. Twenty-one litterbags were placed along a line transect in each plot, ca. 30 cm apart. In the NT plots litterbags were held at the surface with nails. Bags in the CT plots were placed in a vertical slit cut to 15 cm depth with a spade. Three replicate bags from each subplot were randomly removed on each of seven sample dates beginning in December and continuing through May, at 1, 3, 6, 9, 12, 16 and 20 weeks after they were first placed in the field. Litterbags were immediately oven dried (50 °C) after removal from the field. Field samples were gently picked free of extraneous soil and weighed. Sub-samples of the original materials, traveler bags and field bags, were combusted in a muffle furnace at 500 °C to obtain ash free dry weight (AFDW). A correction was made for the AFDW of the initial field bag weights. AFDW was used to obtain the percent mass remaining over time (AFDW final/AFDW initial × 100). Finely ground sub-samples (ball milled) were analyzed for C and N by combustion on a Carlo Erba CNS autoanalyzer (Milan, Italy). The Carlo Erba output gave percent C (%C), percent N (%N) and the C:N ratio with 0.1% variation. Total C and total N of the litter was calculated by multiplying %C and %N by the final weights obtained prior to ashing.

Statistical analyses were performed with the SAS software (SAS, 1998). The average values of %C, %N, ratio C:N, total C, total N, and % mass remaining for the three replicate bags obtained on each date and within each plot were analyzed. The data were fit to a general linear model for analysis of split plots:

$$y_{ijkl} = \mu + \tau_i + \alpha_j + (\tau a)_{ij} + e_{ijk} + \beta_l + (\tau\beta)_{il} + (\alpha\beta)_{jl} + (\tau\alpha\beta)_{ijk} + e_{ijkl},$$

where, τ, α, and β, represent tillage, cotton type, and time, and the error for the whole plot (e_{ijk}) was determined from the value for the replicated field plot nested within the tillage × cotton interaction. The *F*- and *P*-values for the type III sums of squares and those modified by the appropriate error term (tillage, cotton type, and tillage × cotton interaction) are reported.

3. Results

Percent mass of litter (AFDW) remaining decreased significantly in all treatments over time (Fig. 1). Over 55% of the litter material was decomposed in CT after 20 weeks of incubation, leading to a decomposition rate that was twice as fast as that in the NT plots. Percent mass loss was significantly affected by tillage and time, although there was a significant interaction effect that was due to similar decomposition rates between CT and NT in the first six weeks (Fig. 1, Table 1). Although RR+Bt cotton material appeared to lose mass at a greater rate under both tillage regimes than the RR−Bt material (Fig. 1), this effect on decomposition dynamics was not significant (Table 1).

Total N content of the cotton litter declined with time. However, the proportion of material by mass that was N (%N) did not change significantly (Fig. 2A and B). Significant decrease in total N with time was dependent on tillage treatment where decomposition dynamics were similar in the first nine weeks (Tables 1,Fig. 2A). Cotton type had no significant effect on N dynamics (Table 1). The averages ± S.D. of %N across the incubation for NT(RR−Bt), NT(RR+Bt), CT(RR−Bt) and CT(RR+Bt) were, 1.16 ± 0.08%, 1.21 ± 0.04%, 1.12 ± 0.12% and 1.12 ± 0.08%, respectively.

Total C decreased with time, with greater loss in the CT (Fig. 2C). In the NT, %C did not change significantly with time, however, in CT plots, %C decreased initially but was not substantially different from initial

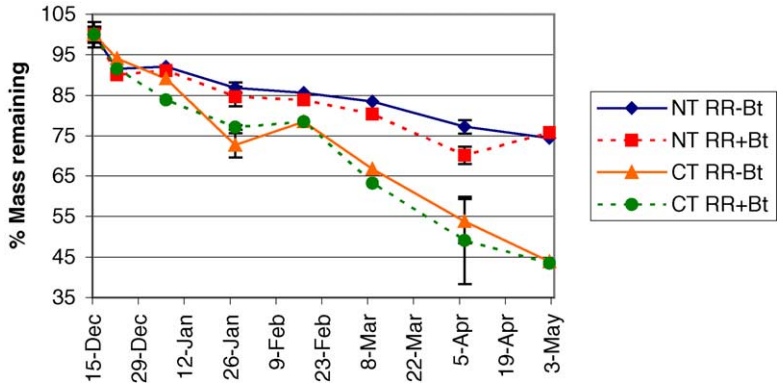

Fig. 1. Percent mass remaining (AFDW) of cotton litter by tillage and cotton type over 20 weeks of decomposition. Bars represent standard deviation, $n = 3$. CT: conventional-tillage; NT: no-tillage; RR+Bt: glyphosate-tolerant *Bt* cotton; RR−Bt: glyphosate-tolerant non-*Bt* cotton.

Table 1
F-values for % mass remaining, total N, %N, total C, %C, and C:N ratio by the factors and interactions of time, tillage and cotton

	d.f.	Mass remaining (%)	Total N	%N	Total C	%C	C:N
Time	6	90.35****	19.89****	1.31	67.22****	3.2**	0.48
Tillage	1	56.31****	14.8**	2.50	73.61****	36.27***	1.54
Cotton	1	1.40	0.1	0.23	1.64	0.27	0.78
Time × tillage	6	18.66***	7.0***	0.81	16.93***	4.38**	2.26*
Tillage × cotton	6	0.02	0.2	0.38	1.33	1.73	0.04
Cotton × time	6	0.82	1.8	2.01*	0.7	0.66	1.72
Cotton × tillage × time	6	0.51	0.1	0.45	0.7	0.77	0.5

* $P < 0.1$.
** $P < 0.01$.
*** $P < 0.001$.
**** $P < 0.0001$.

values at the end of the incubation (Fig. 2D). Both total C and %C were significantly affected by time and tillage, although the effect on total C was dependent more on time whereas the effect on %C was influenced more by tillage; cotton type did not significantly affect C dynamics (Table 1).

The C:N ratio of the decomposing cotton residue fluctuated between 30 and 40 for all treatments with no consistent pattern over time (Fig. 3). There were no strong effects of time, tillage or cotton on C:N (Table 1); the overall model for the test of significance had a *P*-value of 0.0399.

4. Discussion

Differences in decomposition dynamics with *Bt*-modification of this cotton variety were not established. There was no substantial difference in nutrient content of the material between the two gene-modified types tested. Lack of resource quality differences measured by C and N content were not unexpected. Ridley et al. (2002) showed no significant differences in the nutritional profile of glyphosate-tolerant corn to conventional corn. Coviella et al. (2000) showed no significant differences in N content of *Bt* cotton from non-*Bt* cotton. However, Saxena and Stotzky (2001b) showed a significant difference in the lignin content of *Bt* corn from non-*Bt* corn. We did not confirm an alteration in resource quality of the gene-modified types expressing glyphosate tolerance or *Bt* from the unaltered parent line. Our findings suggest that resource quality differences in relation to the genetic modification of plants might not be reliably evaluated by nutrient content (C and N) alone.

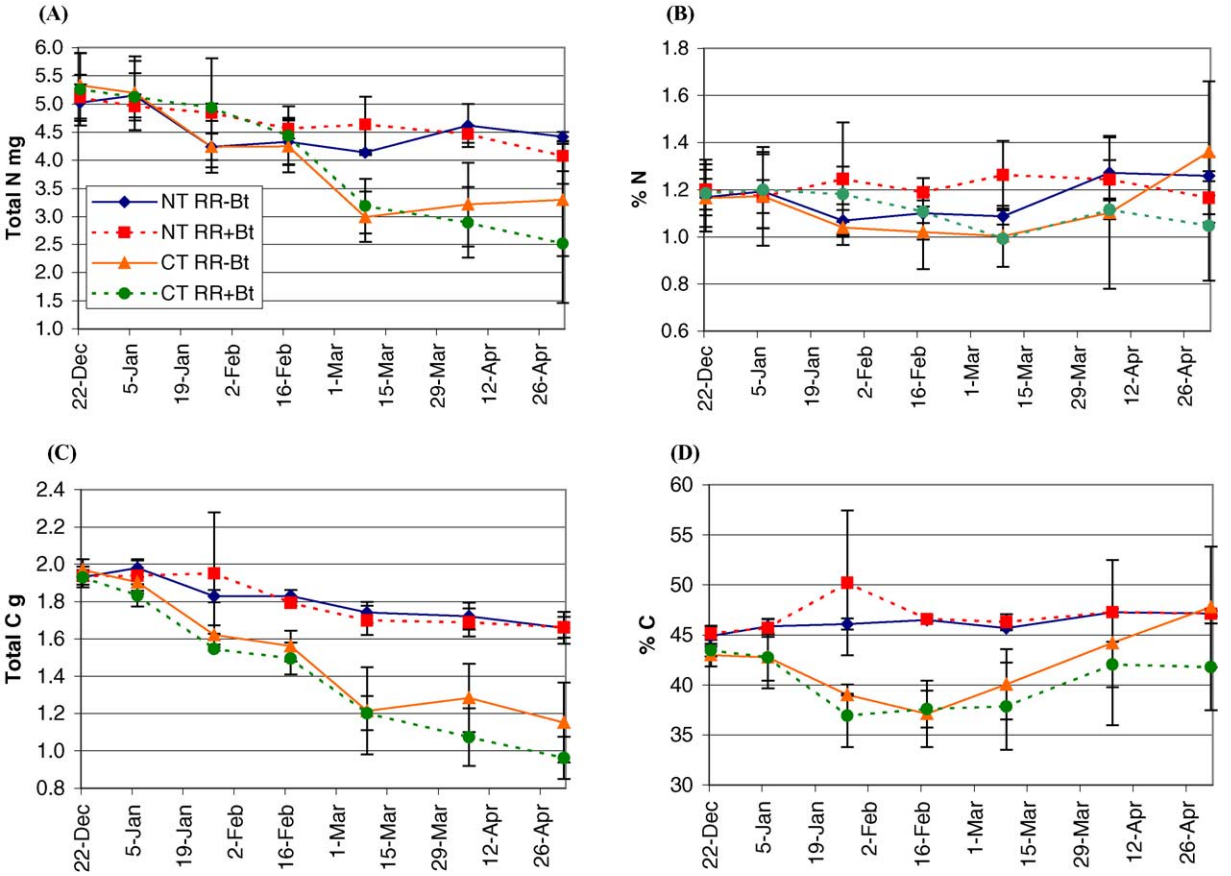

Fig. 2. Nutrient content of cotton litter by tillage and cotton type over 20 weeks of decomposition. (A) Total N (mg) remaining; (B) percent N; (C) total C (g) remaining; (D) percent C. Bars represent standard deviation, $n = 3$. See Fig. 1 for abbreviations.

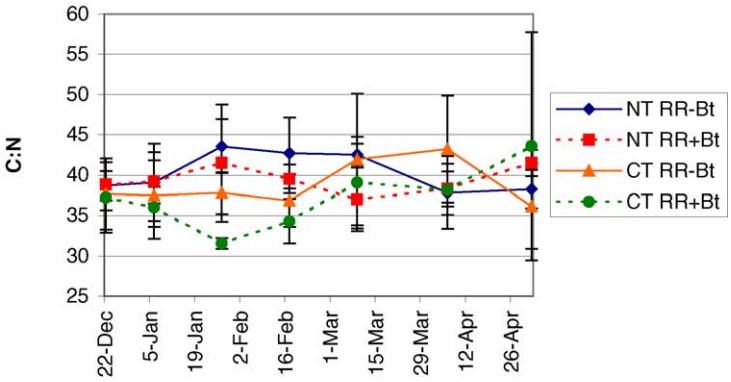

Fig. 3. Carbon to nitrogen ratio of litter by tillage and cotton type over 20 weeks of decomposition. Bars represent standard deviation, $n = 3$. See Fig. 1 for abbreviations.

The difference in decomposition between the two tillage regimes was expected since the litterbags were buried in the conventional-tillage plots, allowing them full contact with soil, and the microbes and fauna contained within. Decomposition rates for many different residue types have consistently been faster in CT than NT at this site (House et al., 1984; Beare et al., 1992). Faster decomposition of buried litter in comparison to surface litter is often associated with higher water content and greater densities of decomposer organisms (Beare et al., 1992). Compared to surface placement, litter burial resulted in faster decomposition of canola straw (Blenis et al., 1999), corn, soybean, wheat, grain sorghum, and cotton residues (Ghidey and Alberts, 1993).

Increase in total N, expressed by percent of initial N, as materials decompose has been documented in various residue types. Increase in %N-remaining was observed in surface and subsurface decomposing rye (Beare et al., 1992). In buried wheat residues %N-remaining was above 100% the first 20 days of incubation (Cookson et al., 1998). N content of wheat was above initial values for the majority of a six-month decomposition study, however in lupin residues %N-remaining dropped below 100% after the first three months (van Vliet et al., 2000). Holland and Coleman (1987) proposed that bridges of fungal hyphae lead to increased N in decomposing wheat straw after 14 months of incubation. Frey et al. (2000) determined that fungal translocation was a direct mechanism for N immobilization in decomposing surface organic material. Although fungal hyphae were noted in litterbags collected from both NT and CT treatments we did not observe a significant change in %N, or an increase in total litter N. Lack of an effect on N dynamics attributable to fungal translocation could be due to the bacterial domination of biomass and metabolic activity (minor fungal contribution) through the winter–spring cropping season at HSB (Hendrix et al., 1987).

As decomposition proceeds, materials with a low C:N may be readily utilized by organisms leaving behind more recalcitrant materials with a greater C:N such as lignin. However, translocation of N into decomposing material can maintain or decrease C:N (Holland and Coleman, 1987; Beare et al., 1992). The C:N ratio of decomposing organic matter should change over time, with the ratio most likely to increase as recalcitrant materials remaining have a higher C:N, and decomposition eventually becomes limited by nitrogen, but we did not observe this trend. The change in C over time was similar to the decrease in %C observed in decomposing transgenic tobacco (Donegan et al., 1997). A decrease in %C and a minimal change in %N, which results in a decrease in C:N ratio, was observed in transgenic tobacco expressing the tomato proteinase inhibitor I (pJN3) for resistance to lepidopteran pests (Donegan et al., 1997).

There is a lack of information on decomposition dynamics of cotton residue and description of decomposer communities contributing to its decomposition. Much cotton is still produced under conventional-tillage management and defoliation occurs prior to harvest. Defoliation chemicals might also affect decomposition of cotton residue. Saxena and Stotzky (2001a) confirmed that *Bt* toxin was available to organisms through root exudation or ingested plant material. However, no apparent toxic or reproductive effect of *Bt* expression in cotton or corn has been established for single species of collembola, oribatida, and oligochaeta or numbers of bacteria, fungi, nematodes and protozoa (Yu et al., 1997; Saxena and Stotzky, 2001a). The collembola, oribatid and earthworm species so far investigated do not occur at the HSB field site. Nonetheless, current research has not established that transgenic crops will have no effect on soil biological activity over the long-term. We do not know what the potential long-term effect alteration in taxonomic diversity of microflora established in the rooting zone of transgenic crops (Donegan et al., 1995; Di Giovanni et al., 1999; Siciliano and Germida, 1999) will be. Although previous studies have documented that GMOs affect taxonomic diversity of microflora, the present study has established that effects on ecosystem function between the RR−Bt and RR+Bt crops under two tillage regimes was not apparent after one season of cropping. However, after consecutive cotton crops at the HSB field site, we have found an accumulation of the *Bt*-protein and/or its breakdown products at the 15–30 cm depth (Hunter et al., unpublished data).

Although we did not find differences in decomposition dynamics due to *Bt* expression in glyphosate-tolerant cotton, we did confirm that tillage and litter placement have considerable effect on rates of decomposing material. Increase in N and change in

C:N ratio, which are typical findings of many decomposition studies, were not confirmed by our study. Further experiments are needed to assess decomposer community dynamics in cotton and other transgenic crops after continuous use of these crops in the field, and to establish better measures of the influence of genetic modification on resource quality.

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